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Cascade of Fever Production in Mice Infected With Influenza Virus

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The cascade of fever production in influenza was studied. To analyse fever production in a murine model, we selected DBA/2 mice that have the highest susceptibility in fibrile responses among seven mouse strains. Intranasal influenza infection- and interferon (IFN)-induced fever production was studied in this mouse model. Fever was induced prominently on day 2 after influenza infection and IFN activity was also increased in serum. Only the level of interleukin (IL)-1α, an endogenous pyrogen, rose markedly in serum among cytokines (IL-1α, IL-2, IFN-γ, and tumor necrosis factor-α) examined. Fever was induced 14 hr after intraperitoneal IFN-α treatment and IL-1α level rose significantly in the serum of the IFN-α-treated mice as compared with that of untreated mice. Fever production was significantly suppressed by treatment with anti-IFN-α/β or anti-IL-1α antibody in infected mice and the former signficantly suppressed responsive IL-1α production, indicating that elevated IFN activity induced IL-1α production and subsequently fever production in infected mice. The activity of cyclooxygenase (COX) that produces prostaglandin (PG)E_2 was significantly augmented in the brain of infected mice on day 2 after infection. Fever production was suppressed by the inhibition of COX activity with aspirin, although IL-1α level was maintained at the elevated level. Therefore, influenza infection in mice turned on the following cascade for fever induction: IFN production, IL-1α production, elevated COX activity, and PGE_2 production. We elucidated the relationship among IFN activity, IL-1α production and COX activity and demonstrated the cascade of fever production in influenza infection.

KEY WORDS: influenza virus, fever, interferon, cytokines, interleukin-1α, cyclooxygenase activity

INTRODUCTION

Fever is one of the major symptoms in the acute phase of influenza but the precise mechanism of fever production is not yet clearly understood. This infection has been shown experimentally to induce fever as well as cytokines such as interferon (IFN) and interleukin (IL)-1 [Bernheim et al., 1980; Dinarello, 1981; Hennet et al., 1992; Murphy et al., 1973; Taylor et al., 1989], and these cytokines are thought to be endogenous pyrogens [Blatteis, 1992; Dinarello et al., 1988]. The interaction of circulating endogenous pyrogens with sensory elements in the organum vasculosum laminae terminalis (OVLT) is a critical step in initiating febrile response which is mediated by a metabolic pathway involving the generation of prostaglandin E_2 (PGE_2) [Ballou et al., 1992; Blatteis, 1992; Hashimoto et al., 1991; Saper and Breder, 1992]. However, a series of processes initiating febrile response after influenza infection is not clearly understood.

We have been using experimental animal models to develop a new treatment of viral infection [Kurokawa et al., 1993a, 1993b, 1995; Nagasaka et al., 1995]. In the series of studies using a mouse model, we analysed the process of fever production during influenza virus infection. This model has been used as a human model for influenza virus infection since influenza virus produces typical pneumonia [Tashiro et al., 1987a and 1987b]. The pneumonia observed in the infected mice is caused by host immune response against influenza virus [Hennet et al., 1992; Hurd and Heath, 1975; Sullivan et al., 1976] and histopathological findings in infected murine pulmonary tissues are similar to the pathological changes during human infection [Hert et al., 1962]. Based on this infection model, we developed a fever production system using a mouse strain with the high susceptibility to IFN among seven mouse strains, because IFN is known to induce fever [Dinarello et al., 1988]. In this mouse strain, influenza virus infection and IFN

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induced fever production but anti-IFN antibody and aspirin reduced the fever. The elevation of rectal temperature resulted from the increased PGE_2 production by enhanced activity of cyclooxygenase (COX) [Dinarello et al., 1988]. These results are consistent with those of immunological parameters related to fever production as reported earlier [Dinarello et al., 1988; Insel, 1992; Saper and Breder, 1992]. Although this murine model may not be the best model for human, it would be suitable for evaluating fever production upon infection with influenza virus. We examined the role of IFN and IL-1α in fever production together with the inhibitory activity of anti-IFN and anti-IL-1α antibodies in the murine model.

MATERIALS AND METHODS

Cells and Viruses

Mouse L929 cells were grown and maintained in Eagle's minimum essential medium (MEM) supplemented with 5% and 2% heat-inactivated calf serum, respectively. Influenza virus [A/PR/8/34 (H1N1)] was propagated in the lung of mice by intranasal infection. The lungs of infected mice were removed and homogenised in phosphate-buffered saline (PBS). The homogenate was centrifuged at 3,000 rpm for 15 min and then the supernatant was stored at −80°C as an inoculum. Vesicular stomatitis virus (VSV, New Jersey strain) was provided from the National Institute of Animal Health, Japan and used for the IFN assay [Nagasaka et al., 1995].

Fever Production Model by IFN

Since IFN is a factor that induces fever [Dinarello et al., 1988], we examined the rectal temperature in various mouse strains treated with IFN-α to select the most suitable mouse strain as a fever production model. Female DBA/2 Cr, ICR, C57BL/6 Cr, BALB/c Cr, A/J, C3H/He and ddY strains (4 to 6-week old, 16–18 g) purchased from Sankyo Labo Service Co., Ltd., Japan, were used. Recombinant mouse IFN-α (5×10^4 IU/mouse, Gibco BRL, USA) or PBS was intraperitoneally administered to these mice. Rectal temperature was monitored by a thermometer (Sato Keiryoki MFG, Co., Ltd, Japan) every 3 to 4 hr after IFN-α administration.

Influenza Virus Infection Model in Mice

We examined macroscopically the development of hemorrhagic lesions in the lungs of infected mice to confirm whether influenza virus infection causes pneumonia in mouse strains used as a model for fever production. Female ICR or DBA/2 Cr mice (5 or 6-week-old, respectively, 16–18 g) were infected intranasally or mock-infected with 600–800 plaque forming units (PFU) of influenza virus under ether anaesthesia. The development of consolidation of lungs was observed and scored according to the method of Ginsberg and Horsfall [1952] with modifications. The following scores were used: 0, no consolidation in a lobe; score 1, consolidation of less than 50% of surface area of a lobe; score 2, consolidation of more than 50% of surface area of a lobe. The score of each lobe was added and the total was defined as the consolidation score of a mouse lung with five lobes. Also, the lungs were examined histopathologically as described previously [Nagasaka et al., 1995].

Determination of Serum IFN Activity

Sera were prepared from four to five mice in each group on day 2 postinfection and examined for IFN activity by the plaque reduction assay of VSV in mouse L929 cells as reported previously [Nagasaka et al., 1995]. Briefly, mouse L929 cells were grown in 12-well plates at 37°C for 24 hr. The cells were pretreated with medium containing serially diluted serum for 24 hr at 37°C. The culture fluids were removed and the cultures were infected with 50 PFU of VSV for 1 hr. Then the cells were overlaid with nutrient methylcellulose medium. The infected cultures were incubated for 3 days at 37°C. The number of plaques was counted after fixation and staining. Serum IFN level was expressed as the IFN international unit (IU) by comparison with standard recombinant murine IFN-α (10^9 IU/mg, Gibco BRL, USA).

Determination of Cytokines in Serum or Bronchoalveolar Lavage Fluid

The levels of cytokines in the bronchoalveolar lavage fluid of lungs or serum were examined by an enzymelined immunosorbent assay (ELISA). For the preparation of bronchoalveolar lavage fluid of lungs, the thorax of mouse was opened and then whole lungs were lavaged with 1 ml of MEM using tracheal cannula. The bronchoalveolar fluid was aspirated and instilled into the trachea twice. The bronchoalveolar fluid collected was centrifuged at 2,000 rpm for 10 min and the supernatants were stored at −20°C until their cytokines analysis. Cytokine levels in the bronchoalveolar lavage fluid or serum were determined using ELISA kits for mouse IL-1α, IL-2, tumor necrosis factor-α (TNF-α), and IFN-γ (Genzyme, USA) according to the manufacturer's instructions.

Influenza Virus Infection Model for Fever Induction in Mice

Fever production was examined mainly in DBA/2 Cr mice infected with influenza virus. Infection was performed as described above. Aspirin (26 or 80 mg/kg/day) or water was administered orally to mice immediately and approximately at 8 hr interval after infection. Recombinant mouse IFN-α was intraperitoneally administered to mice to evaluate fever production induced by IFN treatment as described above. Anti-IL-1α (50 μg/day, Paesel and Lorei, Germany) or anti IFN-α/β antibody (5,000 NIH U/day, Advanced Magnetics Inc., USA) was administered intravenously to infected mice once on a day before and once daily after infection to determine the roles of IL-1α and IFN in fever production. Rectal temperature was monitored by a thermometer (Sato Keiryoki MFG, Co., Ltd, Japan) periodically as indicated in the text.

Assay for COX Activity

COX activity in the brain of mice was examined to evaluate the production of PGE_2 and fever. The brain of mice was removed on day 2 after infection and homogenized in 50 mM Tris HCl, pH 8.0. Microsome fractions were prepared from the homogenates. COX activity in the fractions was determined by the radioimmunoassay (NEN Research Products, USA) of PGE_2 which was produced from arachidonic acid added in the reaction mixtures of the assay kit for COX activity (Paesel and Lorei, Germany) according to the manufacturer's instructions. Amounts of protein in the microsome fractions were determined by the Coomassie Brilliant blue R protein assay (Bio-Rad Laboratories, USA) and COX activity was expressed as the amounts of PGE_2 per the amounts of protein.

Statistical Analysis

Student's t-test was used to evaluate the significance of differences between two groups in rectal temperatures, the concentrations of cytokines in the bronchoalveolar lavage fluid and serum, IFN activities and COX activities. A P value of less than 0.05 was statistically defined as significant.

RESULTS

Fever Production by IFN in Mice

Influenza infection induces IFN which results in fever production [Dinarello et al., 1988]. Thus fever production was examined in seven kinds of mouse strains to find a strain with high susceptibility to IFN-α in fever production. When the rectal temperature was examined in DBA/2 Cr, ICR, C57BL/6 Cr, BALB/c Cr, A/J, C3H/He, and ddY strains after IFN-α treatment, DBA/2 Cr, ICR, C57BL/6 Cr, BALB/c Cr, and C3H/He strains significantly responded to IFN-α (data not shown). Fever was the most prominent in DBA/2 Cr strain among them which developed 7 to 20 hr after IFN-α treatment (Fig. 1). Therefore DBA/2 Cr mice were mainly used for the analysis of fever production in this study.

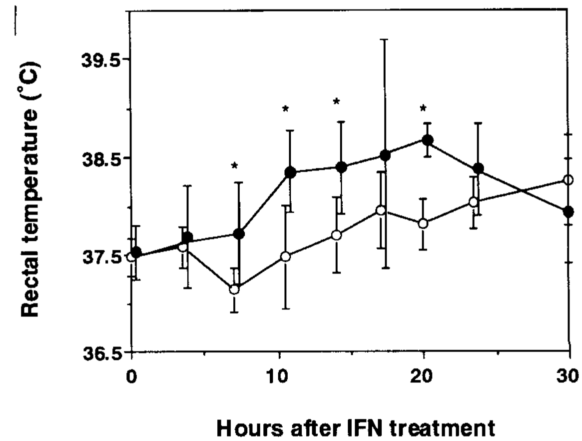

Fig. 1. Effect of IFN-α treatment on the rectal temperature of DBA/2 Cr mice. DBA/2 Cr mice were treated intraperitoneally with recombinant IFN-α (●) or PBS (○) and rectal temperature of 10 mice in each group was monitored every 3 to 4 hr after IFN-α treatment. $^*P < 0.05$ vs. PBS-treated group by the Student's t-test.

TABLE I. Development of Lung Consolidation in Mice Infected With Influenza Virus*

Mouse	Exp. No.	Days after	Consolidation score (mean ± SD)
ICR			
	1	2	0.0
		4	3.3 ± 1.5
		6	8.0 ± 2.0
		8	10.0 ± 0.0
		10	9.0 ± 1.4
	2	2	0.0
		4	5.3 ± 0.6
		6	7.7 ± 0.6
	3	2	0.0
		4	3.7 ± 0.6
		6	8.7 ± 0.6
DBA/2	1	2	0.0
		4	5.0 ± 0.0
		6	5.7 ± 3.2

*The development of lung consolidations was examined in infected mice. The score of consolidation was defined as described in the text. Fifteen mice were used in each group and three or five mice were used for scoring the development of consolidation on the indicated days.

Influenza Virus Infection Model in Mice

When DBA/2 Cr or ICR mice were infected intranasally with influenza virus, the consolidation of lungs was observed in the infected mice after day 4 postinfection (Table I). Histopathological examination of lungs also showed the development of pneumonia. Microscopically, the pathologic changes of lungs became evident on day 4 after the intranasal instillation of influenza virus, and progressed thereafter. The epithelium of bronchi and bronchioles showed inflitration of inflammatory cells and necrosis, and was overlaid by mucopurulent materials. Plugging of some bronchioles resulted in collapse of lung parenchyma which was accompanied by congestion, local haemorrhages, perivascular and intralobular edema, and infiltration with inflammatory leukocytes. Nine or all of 10 infected mice died in each group until 10 days after infection. Influenza virus infection was confirmed to cause pneumonia in our infection model in mice.

Production of Cytokines in Infected Mice

Production of cytokines including IFN as immune mediators or pyrogenic factors were examined in mice infected with influenza virus. IFN activity was significantly higher in the serum of infected mice (172.6 ± 63.6 IU) than in uninfected mice (53.3 ± 3.8 IU) on day 2 after infection ($P < 0.05$ by the t-test). When the concentrations of cytokines were determined in serum of infected mice, the concentrations of IL-1α and IL-2 increased on day 2 postinfection, 3.0–4.4 folds-increase (Table III) and 14.7 folds-increase (data not shown),

TABLE II. Amounts of IL-1α in the Bronchoalveolar Lavage Fluid of Lungs and Serum From Mice Infected With Influenza Virus*

Expt. No.	Days after infection	IL-1α (pg/ml) Uninfected mice	Infected mice
1 Serum	2	38.8 ± 22.8	172.0 ± 79.6
	4		59.2 ± 25.6
	6		27.2
Lavage	2	13.0	152.6
	4		62.0
	6		39.6
2 Serum	2	40.4 ± 11.6	122.4 ± 18.8
	4	16.4 ± 10.8	46.4 ± 5.2
	6	24.0	30.8 ± 16.4
Lavage	2	7.6 ± 13.0	65.2 ± 23.6
	4	6.4 ± 5.8	45.6 ± 40.4
	6	0.0 ± 0.0	16.0 ± 14.0

*IL-1α levels in the bronchoalveolar lavage fluid of lungs and serum were examined as described in the text. Two to three mice were used in each group. Results are expressed as means ± SD.

respectively, but the concentrations of TNF-α and IFN-γ were not affected by the infection (0.8–1.5 folds-increase, data not shown). In the bronchoalveolar lavage fluid of lungs of infected mice, the concentrations of IL-1α, TNF-α, and IFN-γ increased on days 2 to 6 postinfection as compared with those in uninfected mice (Table III) but IL-2 level was not affected by infection (data not shown). The increase of IFN activity and IL-1α level, which are known as endogenous pyrogens [Insel, 1992; Saper and Breder, 1992], was systemically prominent among cytokines examined.

Relationship Between Fever and IL-1α Production

Fever production was examined in DBA/2 Cr mice infected with influenza virus. Fever developed within days 1 and 2 after infection (data not shown). Such mode of fever was also confirmed in infected ICR mice. When fever was produced prominently on day 2 after infection (44–52 hr) and 14 hr after IFN-α treatment, IL-1α levels rose significantly in serum of mice infected with influenza virus and treated with IFN-α (Table III). Fever production correlated with the elevated IL-1α level. The high level of responsive IL-1α production in serum was observed in aspirin-treated mice but fever was significantly decreased.

Effects of Anti-IFNα/β and Anti-IL-1α Antibodies on the Production of IL-1α and Fever

Effects of anti-IFN-α/β and anti-IL-1α antibodies on fever production were examined in influenza virus-infected mice to clarify the roles of IFN-α/β and IL-1α. Both anti-IFN-α/β and anti-IL-1α antibodies significantly reduced fever (Table IV). Anti-IFN-α/β antibody reduced responsive IL-1α production significantly ($P < 0.05$) and the basal level of IL-1α was observed. Therefore, it was confirmed that IFN-α treatment induced the production of IL-1α and fever in mice. The reduction of fever by anti-IL-1α antibody treatment was also confirmed in mice infected with influenza virus. Influenza virus infection turned on IL-1α production induced by IFN production and the suppression of responsive IL-1α production by anti-IFN-α/β or anti-IL-1α antibody resulted in defervescence.

COX Activity in Infected Mice

COX activity increases and subsequently PGE_2 production is augmented, resulting in fever production, [Dinarello et al., 1988]. Thus, COX activity is resposible for fever production. The activity was compared in the brain of infected mice treated with and without aspirin that inactivates COX activity in the arachidonic acid-cascade [Bodel et al., 1973; Clark and Moyer, 1972; Flower and Vane, 1972; Insel, 1992]. As shown in Table V, aspirin significantly reduced COX activity in the brain which was lower than that in mock-infected mice. However, COX activity increased significantly in infected mice as compared with mock-infected mice. Influenza infection augmented COX activity.

DISCUSSION

We used an intranasal influenza virus infection model in mice to elucidate the cascade of fever production. This intranasal infection with PR8 strain causes pneumonia as reported elsewhere [Tashiro et al., 1987a and 1987b], and we confirmed this in our infection models of ICR and DBA/2 Cr mice. Fever was induced before the development of severe pneumonia (Table I), and the fever in the early stage of infection resembled that in influenza infection in humans.

Intranasal influenza infection in mice causes cellular infiltration of the respiratory tract and the development of pneumonia results from pathological damage caused by an immune response against influenza virus infection [Hennet et al., 1992; Hurd and Heath, 1975; Sullivan et al., 1976]. In our study, the levels of IL-1α, TNF-α, and IFN-γ rose markedly in the bronchoalveolar lavage fluid of lungs after infection but the levels of TNF-α and IFN-γ were not affected in serum by infection. This indicates that strong inflammation might occur locally in the lungs of infected mice. Among the cytokines examined, only the level of IL-1α, as an endogenous pyrogen, rose locally and systematically in an early stage of pneumonia in the infected mice (Table II). Lung-resident cells, and possibly alveolar macrophages that produce IL-1α, are suggested to participate actively in the onset of the inflammatory response [Hennet et al., 1992]. IL-1α is an immune mediator and produced only in the early stage after infection in the lungs of infected mice [Hennet et al., 1992]. Therefore the local production of IL-1α may be due to cellular infiltration in lungs of infected mice. The level of IL-1α rose in serum prominently as compared with TNF-α. Since circulating endogenous pyrogens are suggested to interact with OVLT and induce febrile response [Blatteis, 1992; Hashimoto et al., 1991; Saper and Breder, 1992], IL-1α may be a possible endogenous pyrogen that initiates fever in influenza infection.

We developed a model for fever production in mouse strains with high susceptibility to IFN, since IFN in-

TABLE III. Rectal Temperature and IL-1α Levels of Mice Infected With Influenza Virus or Treated With IFN-α*

Treatment		Rectal temperature (°C)	IL-1α (pg/ml) in serum
Influenza virus infection			
Mock-infected	water (n = 7)[a]	37.0 ± 0.2[b]	28.2 ± 12.1[c,d]
Infected	water (n = 10)	37.5 ± 0.1	52.8 ± 18.9
	aspirin (n = 7)	37.2 ± 0.3[c]	58.7 ± 24.0
IFN-α treatment			
PBS-treated	water (n = 5)	36.9 ± 0.2[b]	33.6 ± 10.5[d,e]
IFN-α-treated	water (n = 5)	37.8 ± 0.2	93.6 ± 31.5
	aspirin (n = 5)	37.0 ± 0.3[e]	119.3 ± 51.8

*Rectal temperature and IL-1α levels of DBA/2 Cr mice infected with influenza virus or treated with IFN-α were compared in water- and aspirin (26 mg/kg/day)-treated mice as described in the text. Rectal temperature was measured on day 2 after infection and at 14 hr after IFN-α-treatment, and the same, blood samples were collected and serum IL-1α levels were examined by ELISA.
[a]Number of mice used in each group.
[b]$P < 0.001$ vs. infected mice or IFN-α-treated mice with water administration.
[c]$P < 0.05$ vs. infected mice with water administration.
[d]$P < 0.05$ vs. infected mice or IFN-α-treated mice with aspirin administration.
[e]$P < 0.01$ vs. IFN-α-treated with water administration.

TABLE IV. Effects of Anti-IL-1α or Anti-IFN-α/β Antibody on Rectal Temperatures and IL-1α Levels of Mice Infected With Influenza Virus*

Treatment		Rectal temperature (°C)	IL-1α (pg/ml) in serum
Exp. 1			
Mock-infected	PBS (n = 6)	37.0 ± 0.3[a]	—
Infected	PBS (n = 6)	37.8 ± 0.2	—
	Anti-IL-1α antibody (n = 6)	37.4 ± 0.2[b]	—
Exp. 2			
Mock-infected	PBS (n = 5)	37.0 ± 0.4[c]	43.8 ± 15.0[c]
Infected	PBS (n = 7)	37.6 ± 0.1	82.3 ± 35.6
	Anti-IFN-α/β antibody (n = 7)	37.2 ± 0.1[a]	48.3 ± 19.2[c]

*Effects of anti-IL-1α or anti-IFN-α/β antibody on fever production. Anti-IL-1α antibody, anti-IFN-α/β antibody, or PBS was intravenously administered to influenza virus-infected mice as described in the text. Rectal temperature was monitored on day 2 after infection and serum IL-1α levels were examined by ELISA as described in the text. Figures in parentheses show the number of mice used in each group.
[a]$P < 0.001$ vs. infected mice with PBS administration.
[b]$P < 0.01$ vs. infected mice with PBS administration.
[c]$P < 0.05$ vs. infected mice with PBS administration.

TABLE V. COX Activity in Brain of Influenza Virus-Infected Mice*

Treatment		COX activity in brain (pg of PGE_2/mg of protein)
Mock-infected	water (n = 5)	1.76 ± 0.20[a,b]
Infected	water (n = 5)	2.10 ± 0.04[b]
	aspirin (n = 5)	0.83 ± 0.16[c]

*COX activity in the brain of influenza virus-infected mice was examined in aspirin-treated mice. Mice were infected with influenza virus, and aspirin (80 mg/kg/day) was orally administered as described in the text. The brain of mice was removed under ether anaesthesia on day 2 after infection and homogenised in Tris-buffer. COX activity in the homogenate was determined using the assay kit for the COX activity and a radioimmunoassay as described in the text. Figures in the parentheses show the number of mice used in each group.
[a]$P < 0.05$ vs. infected mice with water administration.
[b]$P < 0.01$ vs. infected mice with aspirin administration.
[c]$P < 0.01$ vs. infected mice with water administration.

duces fever [Dinarello et al., 1988]. Surprisingly, all mouse strains did not respond to IFN same way in fever production although IFN treatment causes unequivocal fever production in patients with chronic hepatitis C. The DBA/2 Cr mouse model was found to be highly susceptible to IFN in fever production. This mouse strain also showed high susceptibility for fever production when infected with influenza virus. Thus using these systems in mice, we analysed the process of fever production and especially elucidated the relation between IFN activated by infection and IL-1α production as an endogenous pyrogen. Figure 2 summarizes the cascade of fever production and the inhibition steps for fever production by aspirin. Fever in mice infected with influenza virus may be caused by the following steps: elevated IFN activity, IL-1α production, augmented COX activity, and PGE_2 production (COX-PGE_2). In these steps, influenza virus infection permitted the increase of IFN activity and

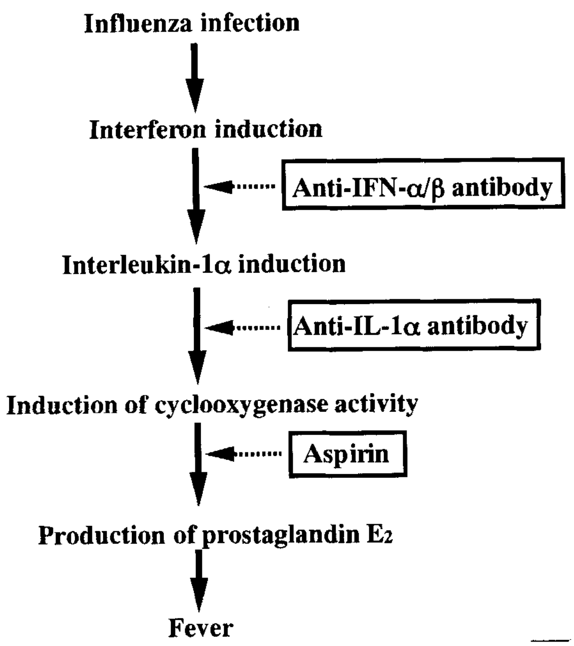

Fig. 2. Cascade of fever induction in influenza and suppression of fever production.

induced responsive IL-1α production and $COX-PGE_2$, resulting in elevation of fever. Aspirin permitted the increase of IFN activity and responsive IL-1α production but reduced $COX-PGE_2$ and fever.

We showed that influenza virus infection increased IFN activity and induced responsive IL-1α production in mice (Tables II, III, and IV). IFN-α treatment also induced the production of IL-1α and fever in mice (Table III). Since IFN is known to induce IL-1α production [Dinarello et al., 1988; Duff and Durum, 1983; Strijbos et al., 1993], IFN activated by infection would induce responsive IL-1α production in influenza virus-infected mice. The suppression of responsive IL-1α production by anti-IFN-α/β antibody in the cascade was prior to that of aspirin that inhibits COX activity (Fig. 2). Even if aspirin reduced COX activity (Table V) and subsequent fever production in infected mice (Table III), IL-1α level was not reduced to the level of uninfected mice. Both of anti-IFN-α/β and anti-IL-1α antibodies reduced fever production in infected mice (Table IV). IL-1α activates $COX-PGE_2$ in hypothalamus and then PGE_2 is induced to produce fever [Ballou et al., 1992; Hashimoto et al., 1991; Saper and Breder, 1992]. Therefore fever may be elevated essentially by the induction of IL-1α production subsequent to IFN production in infected mice. Our findings clarify the relationship among IFN activity, IL-1α production and COX activity and demonstrate the cascade of fever production during influenza infection.

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